```
=> d que l14
L6          154 SEA FILE=HCAPLUS ABB=ON  PLU=ON  COMBINATOR?(2A)BIOSYNTH?
L10          66 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 AND (GLASER OR OLEFIN(2A)ME
                TATHES? OR STILLE OR (FATTY ACID OR POLYKET? OR PEPTIDE OR
                TERPENE OR IOSPREN?)(2A)SYNTHASE OR PARALLEL SYNTH? OR
                SPLIT(2A)POOL OR ENCODING TECHNIQ?)
L11          37 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10 AND ENZYM?
L12          20 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 AND (NONRIBOSOM? OR
                MITSONOBU)
L13          49 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L11 OR L12
L14           4 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L13 AND SUPPORT


=> d ibib abs hitind 1-4 l14

L14  ANSWER 1 OF 4  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:        2003:47973  HCAPLUS
DOCUMENT NUMBER:         138:282019
TITLE:                   Microcystin biosynthesis in Planktothrix: Genes,
                         evolution, and manipulation
AUTHOR(S):               Christiansen, Guntram; Fastner, Jutta; Erhard, Marcel;
                         Borner, Thomas; Dittmann, Elke
CORPORATE SOURCE:        Institut fur biologie (Genetik), Humboldt-Universitat
                         Berlin, Berlin, D-10115, Germany
SOURCE:                  Journal of Bacteriology (2003), 185(2), 564-572
                         CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER:               American Society for Microbiology
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Microcystins represent an extraordinarily large family of cyclic
     heptapeptide toxins that are nonribosomally synthesized by
     various cyanobacteria. Microcystins specifically inhibit the eukaryotic
     protein phosphatases 1 and 2A. Their outstanding variability makes them
     particularly useful for studies on the evolution of structure-function
     relationships in peptide synthetases and their genes. Analyses of
     microcystin synthetase genes provide valuable clues for the potential and
     limits of combinatorial biosynthesis. We have
     sequenced and analyzed 55.6 kd of the potential microcystin synthetase
     gene (mcy) cluster from the filamentous cyanobacterium Planktothrix
     agardhii CYA 126. The cluster contains genes for peptide synthetases
     (mcyABC), polyketide synthases (PKSs; mcyD), chimeric
     enzymes composed of peptide synthetase and PKS modules (mcyEG), a
     putative thioesterase (mcyT), a putative ABC transporter (mcyH), and a
     putative peptide-modifying enzyme (mcyJ). The gene content and
     arrangement and the sequence of specific domains in the gene products
     differ from those of the mcy cluster in Microcystis, a unicellular
     cyanobacterium. The data suggest an evolution of mcy clusters from,
     rather than to, genes for nodularin (a related pentapeptide) biosynthesis.
     Our data do not support the idea of horizontal gene transfer of
     complete mcy gene clusters between the genera. We have established a
     protocol for stable genetic transformation of Planktothrix, a genus that
     is characterized by multicellular filaments exhibiting continuous
     motility. Targeted mutation of mcyJ revealed its function as a gene
     coding for a O-methyltransferase. The mutant cells produce a novel
     microcystin variant exhibiting reduced inhibitory activity toward protein
     phosphatases.
```

CC 3-3 (Biochemical Genetics)
Section cross-reference(s): 4, 6, 7, 10
ST sequence Planktothrix microcystin biosynthesis gene protein **enzyme**
IT 79956-01-7, **Polyketide synthase**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(gene mcyD, sequence homolog; genes, evolution, and manipulation for Planktothrix agardhii microcystin biosynthesis)
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:233726 HCAPLUS
DOCUMENT NUMBER: 135:4489
TITLE: Biosynthesis of hybrid peptide-polyketide natural products
AUTHOR(S): Du, Liangcheng; Shen, Ben
CORPORATE SOURCE: Department of Chemistry, University of California at Davis, Davis, CA, 95616, USA
SOURCE: Current Opinion in Drug Discovery & Development (2001), 4(2), 215-228
CODEN: CODDFF; ISSN: 1367-6733
PUBLISHER: PharmaPress Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 56 refs. The structural and catalytic similarities between **nonribosomal** peptide synthetase (NRPS) and polyketide synthase (PKS) **support** the idea of combining individual NRPS and PKS modules for **combinatorial biosynthesis**. Recent advances in cloning and characterization of biosynthetic gene clusters for naturally occurring hybrid polyketide-peptide metabolites have provided direct evidence for the existence of hybrid NRPS-PKS systems, thus setting the stage to investigate the mol. basis for intermodular communication between NRPS and PKS modules. Reviewed in this article are biosynthetic data pertinent to hybrid peptide-polyketide biosynthesis published up to late 2000. Hybrid peptide-polyketide natural products can be divided into two classes: (i) those whose biosyntheses do not involve functional interaction between NRPS and PKS modules; and (ii) those whose biosyntheses are catalyzed by hybrid NRPS-PKS systems involving direct interactions between NRPS and PKS modules. It is the latter systems that are most likely amenable to **combinatorial biosynthesis**. The same catalytic sites appear to be conserved in both hybrid NRPS-PKS and normal NRPS or PKS systems, with the exception of the ketoacyl synthase domains in hybrid NRPS-PKS systems which are unique. Specific linkers may play a crit. role in communication, facilitating the transfer of the growing intermediates between the interacting NRPS and/or PKS modules. In addn., phosphopantetheinyl transferases with broad carrier protein specificity are essential for the prodn. of functional hybrid NRPS-PKS megasynthetases. These findings should now be taken into consideration in engineered biosynthesis of hybrid peptide-polyketide natural products for drug discovery and development.
CC 16-0 (Fermentation and Bioindustrial Chemistry)
REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:332950 HCAPLUS

TITLE: Bleomycin biosynthesis in Streptomyces verticillus ATCC15003: A model for hybrid peptide and polyketide biosynthesis.
AUTHOR(S): Du, Liangcheng; Sanchez, Cesar; Chen, Mei; Edwards, Daniel J.; Murrell, Jeffrey M.; Shen, Ben
CORPORATE SOURCE: Department of Chemistry, University of California, Davis, CA, 95616, USA
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), ORGN-822. American Chemical Society: Washington, D. C.
CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Polyketides and **nonribosomal** peptides are assembled in a remarkably similar fashion by polyketide synthases (PKSs) from short carboxylic acids and **nonribosomal** peptide synthetases (NRPSs) from amino acids, resp. Cloning and sequence anal. of the 90-kb bleomycin (BLM) biosynthesis cluster from Streptomyces verticillus ATCC15003 revealed both NRPS and PKS genes. By detg. the substrate specificity of individual NRPS and PKS modules, a linear hybrid NRPS/PKS/NRPS model is formulated for the Blm megasynthetase-templated assembly of BLM from nine amino acids and one acetate. These results set the stage for engineering novel BLM analogs by genetic manipulation of the blm biosynthesis genes, **support** the wisdom of combining individual NRPS and PKS modules for **combinatorial biosynthesis**, and lay the foundation to investigate the mol. basis for intermodular communication between NRPS and PKS and the mechanism for bithiazole biosynthesis.

L14 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:188215 HCAPLUS
DOCUMENT NUMBER: 124:226316
TITLE: Antibiotic activity of polyketide products derived from **combinatorial biosynthesis**: implications for directed evolution
AUTHOR(S): Fu, Hong; Khosla, Chaitan
CORPORATE SOURCE: Dep. Chemical Engineering, Stanford Univ., Stanford, CA, 94305-5025, USA
SOURCE: Molecular Diversity (1996), 1(2), 121-4
CODEN: MODIF4; ISSN: 1381-1991
PUBLISHER: ESCOM
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A library of over 100 polyketides, generated via combinatorial cloning of genes encoding subunits of arom. **polyketide synthases**, was screened for mols. capable of inhibiting the growth of gram-pos. bacteria. A total of 26 polyketides, with varying levels of antibiotic activity in filter-disk assays, were purified. Most bioactive polyketides were produced as relatively minor compds. (<1 mg/l), although two major anthraquinones, with yields in the range of 10-100 mg/l, were also identified and structurally characterized. When tested against Bacillus subtilis 168.beta., they were found to cause a 50% redn. in colony-forming units at concns. of 20 and 300 .mu.g/mL, resp. We speculate that many of the minor (and possibly more potent) bioactive polyketides are synthesized via nonspecific **enzymic** modifications of shunt products derived from engineered **polyketide synthase** pathways. If so, then these 'fortuitous' pathways should be amenable to further rationally guided manipulation. Our results **support** the notion that

combinatorial biosynthesis can be used to generate novel, biol. active mols. They also point to the feasibility of designing mutagenesis selection expts. aimed at the directed evolution of org. mols. with desirable pharmaceutical properties.

CC 10-5 (Microbial, Algal, and Fungal Biochemistry)
Section cross-reference(s): 3, 16

ST antibiotic **polyketide** combinatorial **synthase** library; genetic engineering polyketide antibiotic combinatorial library; synthetase polyketide antibiotic genetic engineering

IT Antibiotics
Combinatorial library
Evolution
Genetic engineering
(antibiotic activity of polyketide products derived from **combinatorial biosynthesis** and implications for directed evolution)

IT Polyketides
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(antibiotic activity of polyketide products derived from **combinatorial biosynthesis** and implications for directed evolution)

IT Gene, microbial
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**polyketide synthase** subunit-encoding; antibiotic activity of polyketide products derived from **combinatorial biosynthesis** and implications for directed evolution)

IT 79956-01-7P, **Polyketide synthase**
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(recombinant; antibiotic activity of polyketide products derived from **combinatorial biosynthesis** and implications for directed evolution)

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=> d que
L1                  STR
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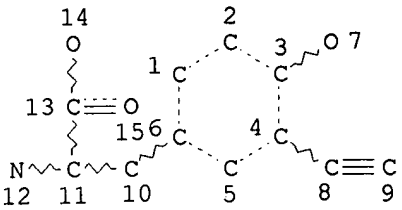

Structure Search

-no overlap with "Key word" search

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CONNECT IS E1  RC AT  12
CONNECT IS E1  RC AT  14
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS  15

STEREO ATTRIBUTES: NONE
L3             2 SEA FILE=REGISTRY SSS FUL L1
L4             2 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L3


=> d ibib abs ind hitstr 1-2

L4   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:         2003:45631  HCAPLUS
DOCUMENT NUMBER:          138:238402
TITLE:                    Use of a Boroxazolidone Complex of 3-Iodo-L-tyrosine
                          for Palladium-Catalyzed Cross-Coupling
AUTHOR(S):                Walker, William H., IV; Rokita, Steven E.
CORPORATE SOURCE:         Department of Chemistry and Biochemistry, University
                          of Maryland, College Park, MD, 20742, USA
SOURCE:                   Journal of Organic Chemistry (2003), 68(4), 1563-1566
                          CODEN: JOCEAH; ISSN: 0022-3263
PUBLISHER:                American Chemical Society
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
OTHER SOURCE(S):          CASREACT 138:238402
AB   Complexation of 3-iodo-L-tyrosine with 9-borabicyclo[3.3.1]nonane (9-BBN)
     provides a convenient substrate for a palladium-catalyzed coupling
     reaction.  The complex is stable to silica gel chromatog. (hexanes/ethyl
     acetate), dil. triethylamine in THF, and potassium fluoride in DMF.  The
     desired product, 3-ethynyl-L-tyrosine, was released from the complex by
     simply dilg. its soln. in methanol with chloroform.  Interestingly, the
     complex remains stable in solns. of either methanol or chloroform
     individually.  None of the synthetic procedures caused racemization of the
     .alpha.-carbon as detected by the consumption of 3-ethynyl-L-tyrosine by
     Crotalus atrox L-amino acid oxidase.
CC   34-2 (Amino Acids, Peptides, and Proteins)
     Section cross-reference(s): 7, 9
ST   iodotyrosine boroxazolidone palladium catalyzed Sonogashira coupling;
```

ethynyl tyrosine prepn Crotalus atrox enzymic digestion

IT Cross-coupling reaction
(Sonogashira; prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT Cross-coupling reaction catalysts
Crotalus atrox
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT 501683-81-4P
RL: BPN (Biosynthetic preparation); RCT (Reactant); BIOL (Biological study); PREP (Preparation); RACT (Reactant or reagent)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT 9000-89-9, L-Amino acid oxidase 9004-07-3, .alpha.-Chymotrypsin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT 58632-95-4, boc-on 70-78-0
RL: BSU (Biological study, unclassified); RCT (Reactant)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT **501683-85-8P**
RL: BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT 143-66-8, Sodium tetraphenylborate 280-64-8, 9-BBN 1066-54-2
RL: RCT (Reactant); RACT (Reactant or reagent)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

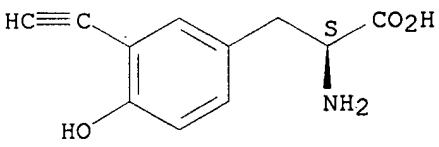
IT 32483-30-0P 71400-63-0P 79677-58-0P 79677-59-1P 501683-86-9P 501683-87-0P 502146-66-9P 502146-67-0P 502146-68-1P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT 502146-64-7P
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

IT **501683-85-8P**
RL: BSU (Biological study, unclassified); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(prepn. of 3-ethynyl-L-tyrosine using a boroxazolidone L-tyrosine complex in the Sonogashira cross-coupling reaction as the key step)

RN 501683-85-8 HCAPLUS
CN L-Tyrosine, 3-ethynyl- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:187678 HCAPLUS
DOCUMENT NUMBER: 128:319017
TITLE: Bioconjugation of peptides by palladium-catalyzed C-C cross-coupling in water
AUTHOR(S): Dibowski, Harald; Schmidtchen, Franz P.
CORPORATE SOURCE: Inst. Org. Chem. Biochem. Tech. Univ. Munchen, Garching, D-85747, Germany
SOURCE: Angewandte Chemie, International Edition (1998), 37(4), 476-478
CODEN: ACIEF5; ISSN: 1433-7851
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The study concerns the palladium-catalyzed cross-coupling of aryl iodides with terminal alkynes. The Castro-Stephens-Sonogashira reaction using a palladium-guanidinophosphane catalysts formed in situ allows the regioselective linking of iodoaryl and alkyne structures; under the extremely mild reaction conditions the native structure and function of the proteins remain unharmed.

CC 9-14 (Biochemical Methods)
Section cross-reference(s): 34

ST bioconjugation peptide palladium catalyzed cross coupling

IT Iodides, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(arom.; bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT Cross-coupling reaction
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT Peptides, reactions
Proteins, general, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT Alkynes
RL: RCT (Reactant); RACT (Reactant or reagent)
(.alpha.-; bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

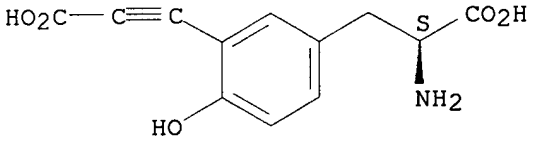
IT 7440-05-3D, Palladium, complexes with guanidinophosphanes, uses 207278-29-3D, complex with palladium 207278-31-7D, complex with palladium
RL: CAT (Catalyst use); USES (Uses)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT 7732-18-5, Water, uses
RL: NUU (Other use, unclassified); USES (Uses)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT 70-78-0 471-25-0, 2-Propynoic acid 619-58-9 23235-01-0 207278-43-1 207278-47-5
RL: RCT (Reactant); RACT (Reactant or reagent)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)

IT **207278-39-5P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)
IT 207278-35-1P 207278-41-9P 207278-45-3P 207278-49-7P
RL: SPN (Synthetic preparation); PREP (Preparation)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)
IT **207278-39-5P**
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(bioconjugation of peptides by palladium-guanidinophosphane catalyzed C-C cross-coupling in water)
RN 207278-39-5 HCAPLUS
CN L-Tyrosine, 3-(carboxyethynyl)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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=> d que
L6          154 SEA FILE=HCAPLUS ABB=ON  PLU=ON  COMBINATOR?(2A)BIOSYNTH?
L10          66 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 AND (GLASER OR OLEFIN(2A)ME
                TATHES? OR STILLE OR (FATTY ACID OR POLYKET? OR PEPTIDE OR
                TERPENE OR IOSPREN?)(2A)SYNTHASE OR PARALLEL SYNTH? OR
                SPLIT(2A)POOL OR ENCODING TECHNIQ?)
L11          37 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10 AND ENZYM?
L12          20 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L6 AND (NONRIBOSOM? OR
                MITSONOBU)
L13          49 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L11 OR L12
L14           4 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L13 AND SUPPORT
L17          13 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L12 AND ENZYM?
L18          42 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L11 OR L17
L21          15 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L18 AND (SUPPORT OR SUBSTRATE
                OR TEMPLATE)
L23          13 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L21 NOT L14

=> d ibib abs hitind l23 1-13

L23  ANSWER 1 OF 13  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:        2003:273243  HCAPLUS
TITLE:                   Computational Approach for Prediction of Domain
                         Organization and Substrate Specificity of
                         Modular Polyketide Synthases
AUTHOR(S):               Yadav, Gitanjali; Gokhale, Rajesh S.; Mohanty,
                         Debasisa
CORPORATE SOURCE:        National Institute of Immunology, New Delhi, 110067,
                         India
SOURCE:                  Journal of Molecular Biology (2003), 328(2), 335-363
                         CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER:               Elsevier Science Ltd.
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   Modular polyketide synthases (PKSs) are large multi-
     enzymic, multi-domain megasynthases, which are involved in the
     biosynthesis of a class of pharmaceutically important natural products,
     namely polyketides.  These enzymes harbor a set of repetitive
     active sites termed modules and the domains present in each module dictate
     the chem. moiety that would add to a growing polyketide chain.  This
     modular logic of biosynthesis has been exploited with reasonable success
     to produce several novel compds. by genetic manipulation.  However, for
     harnessing their vast potential of combinatorial
     biosynthesis, it is essential to develop knowledge based in silico
     approaches for correlating the sequence and domain organization of PKSs to
     their polyketide products.  In this work, we have carried out extensive
     sequence anal. of exptl. characterized PKS clusters to develop an
     automated computational protocol for unambiguous identification of various
     PKS domains in a polypeptide sequence.  A structure based approach has
     been used to identify the putative active site residues of acyltransferase
     (AT) domains, which control the specificities for various starter and
     extender units during polyketide biosynthesis.  On the basis of the anal.
     of the active site residues and mol. modeling of substrates in
     the active site of representative AT domains, we have identified a crucial
     residue that is likely to play a major role in discriminating between
     malonate and methylmalonate during selection of extender groups by this
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domain. Structural modeling has also explained the exptl. obsd. chiral preference of AT domain in **substrate** selection. This computational protocol has been used to predict the domain organization and **substrate** specificity for PKS clusters from various microbial genomes. The results of our anal. as well as the computational tools for prediction of domain organization and **substrate** specificity have been organized in the form of a searchable computerized database (PKSDB). PKSDB would serve as a valuable tool for identification of polyketide products biosynthesized by uncharacterized PKS clusters. This database can also provide guidelines for rational design of expts. to engineer novel polyketides.

CC 7 (Enzymes)

REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:133425 HCAPLUS

DOCUMENT NUMBER: 138:183120

TITLE: Alteration of the **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis

INVENTOR(S): Reeves, Christopher; McDaniel, Robert

PATENT ASSIGNEE(S): Kosan Biosciences, Inc., USA

SOURCE: PCT Int. Appl., 20 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003014312	A2	20030220	WO 2002-US25094	20020806
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003124680	A1	20030703	US 2002-214796	20020807

PRIORITY APPLN. INFO.: US 2001-310730P P 20010807

AB The present invention provides methods for altering the **substrate** specificity of acyltransferase domains of **polyketide synthase** (PKS) **enzymes**, as well as PKS altered by the method, recombinant vectors encoding them, host cells expressing them, and the polyketides so produced. Cassette replacement of acyltransferase (AT) domains in 6-deoxyerythronolide B synthase (DEBS) with heterologous AT domains with different **substrate** specificities usually yields the predicted polyketide analogs. As reported here, however, several AT replacements in module 4 of DEBS failed to produce detectable polyketide under std. conditions, suggesting that module 4 is sensitive to

perturbation of the protein structure when the AT is replaced. Alignments between different modular **polyketide synthase** AT domains and the Escherichia coli **fatty acid synthase** transacylase crystal structure were used to select motifs within the AT domain of module 4 to re-engineer its **substrate** selectivity and minimize potential alterations to protein folding. Three distinct primary regions of AT4 believed to confer specificity for methylmalonyl-CoA were mutated into the sequence seen in malonyl-CoA-specific domains. Two of the regions engineered corresponded to those previously identified by Haydock et al. as important for specificity, whereas the third did not. Region 1, immediately upstream of the highly conserved Gln residue in the active site (Gln-63 in FabD), and region 2, a single amino acid adjacent to the active site serine residue (5ER-92 in FabD) have been implicated in specificity of AT domains based on sequence alignments but have never been directly proven to play a role in specificity. Region 3 is adjacent to a highly conserved histidine residue (His-201) that lies near the active site serine in three dimensional space. Each individual mutation as well as the three in combination resulted in functional DEBSs that produced mixts. of the natural polyketide, 6-deoxyerythronolide B, and the desired novel analog, 6-desmethyl-6-deoxyerythronolide B. The ratios of the polyketide products for each PKS were slightly different, with the region 3 mutations yielding the highest ratio of mCoA to mmCoA incorporation. Prodn. of the latter compd. indicates that the identified sequence motifs do contribute to AT specificity and that DEBS can process a polyketide chain incorporating a malonate unit at module 4. This is the first example in which the extender unit specificity of a PKS module has been altered by site-specific mutation and provides a useful alternate method for engineering AT specificity in the **combinatorial biosynthesis** of polyketides.

IC ICM C12N

CC 7-5 (Enzymes)
Section cross-reference(s): 3, 16

ST deoxyerythronolide B synthase acyltransferase domain mutation **substrate** specificity

IT **Enzyme** functional sites
(active, acyltransferase domain; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT Protein sequences
(alignment; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT Genetic engineering
Molecular modeling
Protein engineering
Protein motifs
(alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT Saccharopolyspora erythraea
Streptomyces
(as host; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT Protein sequences
(engineering **substrate** and polyketide product specificity of

deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT Structure-activity relationship
(**enzyme substrate**; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT Conformation
(protein, anal. of; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT Mutagenesis
(site-directed, substitution; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 79956-01-7, **Polyketide synthase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 357397-19-4, Polyketide acyltransferase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(crystal structure anal. of; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 128172-72-5, 6-Deoxyerythronolide B synthase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 499212-20-3 499212-21-4 499212-22-5
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(mutant amino acid sequence in region 1; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 499212-24-7 499212-25-8 499212-27-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(mutant amino acid sequence in region 2; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 499136-56-0 499212-31-6 499212-33-8 499212-38-3 499212-41-8 499212-44-1
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(mutant amino acid sequence in region 3; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 53428-54-9, 8,8a-Deoxyoleandolide 391902-63-9 497831-78-4 497831-80-8 497831-82-0 497831-84-2
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(prodn. of; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 15797-36-1P, 6-Deoxyerythronolide B
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)

(product; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 524-14-1, Malonyl-CoA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(specificity for, in mutant **enzyme**, **substrate**; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 1264-45-5, Methylmalonyl-CoA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(specificity for, in wild type **enzyme**, **substrate**; alteration of **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutagenesis)

IT 499212-19-0
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(wt amino acid sequence in region 1; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 499212-23-6
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(wt amino acid sequence in region 2; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 499212-29-2
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(wt amino acid sequence in region 3; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

L23 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:664427 HCAPLUS
DOCUMENT NUMBER: 137:334558
TITLE: Utilization of Alternate **Substrates** by the First Three Modules of the Epothilone Synthetase Assembly Line
AUTHOR(S): Schneider, Tanya L.; Walsh, Christopher T.; O'Connor, Sarah E.
CORPORATE SOURCE: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, USA
SOURCE: Journal of the American Chemical Society (2002), 124(38), 11272-11273
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The epothilones, a family of macrolactone natural products produced by the myxobacterial species Sorangium cellulosum, are of current clin. interest as antitumor agents. Inspection of the structure of the epothilones suggests a hybrid polyketide/**nonribosomal** peptide biosynthetic origin, and the recent sequencing of the epothilone biosynthetic gene cluster has validated this proposal. Here we have examd. unnatural

substrates with the first two **enzymes** of the biosynthetic pathway, EpoA and EpoB, to investigate the **enzymic** construction of alternate heterocyclic structures and the subsequent elongation of these products by the third **enzyme** of the pathway, EpoC. The epothilone biosynthetic machinery can utilize serine to install an oxazole in place of a thiazole in the epothilone structure and will tolerate functionalized donor groups from the EpoA-ACP domain to produce epothilone fragments modified at the C21 position. These studies with the early **enzymes** of the epothilone biosynthesis cluster suggest that **combinatorial biosynthesis** may be a viable means for producing a variety of epothilone analogs that incorporate diversity into the heterocycle starter unit.

CC 7-3 (Enzymes)

ST **substrate** specificity epothilone synthetase Sorangium serine polyketide oxazole analog

IT Sorangium cellulosum
Structure-activity relationship
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 252877-37-5, Epothilone synthetase
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(utilization of alternate **substrates** to form oxazole analogs by first three modules of epothilone synthetase assembly line)

IT 23000-14-8P 23012-17-1P 189453-10-9P 198571-09-4P 474123-36-9P 474123-37-0P 474123-38-1P
RL: BPN (Biosynthetic preparation); PRP (Properties); BIOL (Biological study); PREP (Preparation)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 474123-34-7P
RL: BPN (Biosynthetic preparation); PRP (Properties); RCT (Reactant); BIOL (Biological study); PREP (Preparation); RACT (Reactant or reagent)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 1457-58-5P 474123-35-8P 474123-39-2P
RL: BPN (Biosynthetic preparation); PRP (Properties); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 56-45-1, L-Serine, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 70-23-5, Ethyl bromopyruvate 108-24-7, Acetic anhydride 110-71-4, Ethylene glycol dimethyl ether 4530-20-5 19172-47-5, Lawesson's reagent 24720-64-7 35034-22-1 162558-25-0 181954-34-7
RL: RCT (Reactant); RACT (Reactant or reagent)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 20584-70-7P 35150-09-5P 85806-67-3P 89226-13-1P 96929-05-4P 113732-84-6P 139630-91-4P 141029-63-2P 165667-54-9P 474123-40-5P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)

IT 474123-41-6P
RL: SPN (Synthetic preparation); PREP (Preparation)
(utilization of alternate **substrates** to form oxazoles by first three modules of epothilone synthetase assembly line)
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2002:453501 HCAPLUS
DOCUMENT NUMBER: 137:46081
TITLE: Ways of assembling complex natural products on modular **nonribosomal** peptide synthetases
AUTHOR(S): Mootz, Henning D.; Schwarzer, Dirk; Marahiel, Mohamed A.
CORPORATE SOURCE: Fachbereich Chemie/Biochemie, Philipps-University of Marburg, Marburg, 35032, Germany
SOURCE: ChemBioChem (2002), 3(6), 490-504
CODEN: CBCHFX; ISSN: 1439-4227
PUBLISHER: Wiley-VCH Verlag GmbH
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review. **Nonribosomal** peptide synthetases (NRPSs) catalyze the assembly of a large no. of complex peptide natural products, many of which display therapeutically useful activity. Each cycle of chain extension is carried out by a dedicated module of the multifunctional **enzymes**. A module harbors all the catalytic units, which are referred to as domains, necessary for recognition, activation, covalent binding, and optionally modification of a single building block monomer, as well as for peptide-bond formation with the growing chain. A terminal domain releases the full-length peptide chain from the **enzyme** complex. Recent characterization of many NRPS systems revealed several examples where the sequence of the product does not directly-correspond to the linear arrangement of modules and domains within the **enzyme**(s). It is now obvious that these systems cannot, be regarded as rare exceptions of the common NRPS architecture but rather represent more complicated variations of the NRPS repertoire. to increase their biosynthetic potential. In most of these cases unusual peptide structures of the products are obsd., such as structures with side-chain acylation, cyclization involving the peptide backbone and/or side chains, and transfer of the peptide chain onto sol. small-mol. **substrates**. These findings indicate a previously unexpected higher versatility of the modules and domains in terms of both catalytic potential and interaction within the multifunctional protein **templates**. We propose to classify the known NRPS systems into three groups, linear NRPSs (type A), iterative NRPSs (type B), and nonlinear NRPSs (type C), according to their biosynthetic logic. Understanding the various biosynthetic strategies of NRPSs will be crucial to fully explore their potential for engineered **combinatorial biosynthesis**.
CC 16-0 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 7
ST review natural product **nonribosomal** peptide synthetase
IT Natural products
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ways of assembling complex natural products on modular **nonribosomal** peptide synthetases)
IT 115288-50-1, **Nonribosomal** peptide synthetase
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(ways of assembling complex natural products on modular **nonribosomal** peptide synthetases)
REFERENCE COUNT: 104 THERE ARE 104 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:869172 HCAPLUS
DOCUMENT NUMBER: 136:130697
TITLE: Alteration of the **substrate** specificity of a modular **polyketide synthase** acyltransferase domain through site-specific mutations
AUTHOR(S): Reeves, Christopher D.; Murli, Sumati; Ashley, Gary W.; Piagentini, Misty; Hutchinson, C. Richard; McDaniel, Robert
CORPORATE SOURCE: Kosan Biosciences Inc., Hayward, CA, 94545, USA
SOURCE: Biochemistry (2001), 40(51), 15464-15470
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cassette replacement of acyltransferase (AT) domains in 6-deoxyerythronolide B synthase (DEBS) with heterologous AT domains with different **substrate** specificities usually yields the predicted polyketide analogs. As reported here, however, several AT replacements in module 4 of DEBS failed to produce detectable polyketide under std. conditions, suggesting that module 4 is sensitive to perturbation of the protein structure when the AT is replaced. Alignments between different modular **polyketide synthase** AT domains and the Escherichia coli **fatty acid synthase** transacylase crystal structure were used to select motifs within the AT domain of module 4 to re-engineer its **substrate** selectivity and minimize potential alterations to protein folding. Three distinct primary regions of AT4 believed to confer specificity for methylmalonyl-CoA were mutated into the sequence seen in malonyl-CoA-specific domains. Each individual mutation as well as the three in combination resulted in functional DEBSs that produced mixts. of the natural polyketide, 6-deoxyerythronolide B, and the desired novel analog, 6-desmethyl-6-deoxyerythronolide B. Prodn. of the latter compd. indicates that the identified sequence motifs do contribute to AT specificity and that DEBS can process a polyketide chain incorporating a malonate unit at module 4. This is the first example in which the extender unit specificity of a PKS module has been altered by site-specific mutation and provides a useful alternate method for engineering AT specificity in the **combinatorial biosynthesis** of polyketides.
CC 7-5 (Enzymes)
Section cross-reference(s): 16
ST deoxyerythronolide B synthase acyltransferase domain mutation **substrate** specificity
IT **Enzyme** functional sites
(acyltransferase domain; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)
IT Protein engineering
(engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)

IT 9054-54-0, Acyltransferase 128172-72-5, 6-Deoxyerythronolide B synthase
RL: BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); USES (Uses)
(engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)
IT 15797-36-1P, 6-Deoxyerythronolide B 391902-63-9P
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)
(product; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)
IT 524-14-1, Malonyl-CoA 1264-45-5, Methylmalonyl-CoA
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**substrate**; engineering **substrate** and polyketide product specificity of deoxyerythronolide B synthase through site-specific mutations of acyltransferase domain module 4)
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:505089 HCAPLUS
DOCUMENT NUMBER: 135:329041
TITLE: Engineered biosynthesis of novel polyenes: a pimaricin derivative produced by targeted gene disruption in Streptomyces natalensis
AUTHOR(S): Mendes, M. V.; Recio, E.; Fouces, R.; Luiten, R.; Martin, J. F.; Aparicio, J. F.
CORPORATE SOURCE: Institute of Biotechnology INBIOTEC, Leon, 24006, Spain
SOURCE: Chemistry & Biology (2001), 8(7), 635-644
CODEN: CBOLE2; ISSN: 1074-5521
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Background: The post-**polyketide synthase** biosynthetic tailoring of polyene macrolides usually involves oxidns. catalyzed by cytochrome P 450 monooxygenases (P450s). Although members from this class of **enzymes** are common in macrolide biosynthetic gene clusters, their specificities vary considerably toward the **substrates** utilized and the positions of the hydroxyl functions introduced. In addn., some of them may yield epoxide groups. Therefore, the identification of novel macrolide monooxygenases with activities toward alternative **substrates**, particularly epoxidases, is a fundamental aspect of the growing field of **combinatorial biosynthesis**. The specific alteration of these activities should constitute a further source of novel analogs. We investigated this possibility by directed inactivation of one of the P450s belonging to the biosynthetic gene cluster of an archetype polyene, pimaricin. Results: A recombinant mutant of the pimaricin-producing actinomycete Streptomyces natalensis produced a novel pimaricin deriv., 4,5-deepoxypimaricin, as a major product. This biol. active product resulted from the phage-mediated targeted disruption of the gene pimD, which encodes the cytochrome P 450 epoxidase that converts deepoxypimaricin into pimaricin. The 4,5-deepoxypimaricin has been identified by mass spectrometry and NMR following high-performance liq. chromatog. purifn. Conclusions: We have demonstrated that PimD is the epoxidase responsible for the conversion of

4,5-deepoxypimaricin to pimaricin in S. natalensis. The metabolite accumulated by the recombinant mutant, in which the epoxidase has been knocked out, constitutes the first designer polyene obtained by targeted manipulation of a polyene biosynthetic gene cluster. This novel epoxidase could prove to be valuable for the introduction of epoxy substituents into designer macrolides.

CC 10-2 (Microbial, Algal, and Fungal Biochemistry)
Section cross-reference(s): 7

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:428909 HCAPLUS
DOCUMENT NUMBER: 135:177170
TITLE: Assessing the balance between protein-protein interactions and **enzyme-substrate** interactions in the channeling of intermediates between **polyketide synthase** modules
AUTHOR(S): Wu, Nicholas; Tsuji, Stuart Y.; Cane, David E.; Khosla, Chaitan
CORPORATE SOURCE: Departments of Chemistry Chemical Engineering and Biochemistry, Stanford University, Stanford, CA, 94305, USA
SOURCE: Journal of the American Chemical Society (2001), 123(27), 6465-6474
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 6-Deoxyerythronolide B synthase (DEBS) is the modular **polyketide synthase** (PKS) that catalyzes the biosynthesis of 6-deoxyerythronolide B (6-dEB), the aglycon precursor of the antibiotic erythromycin. The biosynthesis of 6-dEB exemplifies the extraordinary **substrate**- and stereo-selectivity of this family of multifunctional **enzymes**. Paradoxically, DEBS has been shown to be an attractive scaffold for **combinatorial biosynthesis**, indicating that its constituent modules are also very tolerant of unnatural **substrates**. By interrogating individual modules of DEBS with a panel of diketides activated as N-acetylcysteamine (NAC) thioesters, it was recently shown that individual modules have a marked ability to discriminate among certain diastereomeric diketides. However, since free NAC thioesters were used as **substrates** in these studies, the modules were primed by a diffusive process, which precluded involvement of the covalent, **substrate**-channeling mechanism by which **enzyme**-bound intermediates are directly transferred from one module to the next in a multimodular PKS. Recent evidence pointing to a pivotal role for protein-protein interactions in the **substrate**-channeling mechanism has prompted the authors to develop novel assays to reassess the steady-state kinetic parameters of individual DEBS modules when primed in a more "natural" channeling mode by the same panel of diketide **substrates** used earlier. Here the authors describe these assays and use them to quantify the kinetic benefit of linker-mediated **substrate** channeling in a modular PKS. This benefit can be substantial, esp. for intrinsically poor **substrates**. Examples are presented where the kcat of a module for a given diketide **substrate** increases >100-fold when the **substrate** is

presented to the module in a channeling mode as opposed to a diffusive mode. However, the **substrate** specificity profiles for individual modules are conserved regardless of the mode of presentation. By highlighting how **substrate** channeling can allow PKS modules to effectively accept and process intrinsically poor **substrates**, these studies provide a rational basis for examg. the enormous untapped potential for **combinatorial biosynthesis** via module rearrangement.

CC 7-4 (Enzymes)

ST **polyketide synthase** module intermediate kinetic channeling; deoxyerythronolide synthase module intermediate kinetic channeling

IT Proteins, specific or class
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)
(ACP (acyl-carrier), thioesters with methylhydroxypentanoate; kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT **Enzyme** kinetics
Michaelis constant
(kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT 128172-72-5, 6-Deoxyerythronolide B synthase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT 354530-30-6DP, thioesters with acyl carrier protein ACP4 354530-31-7DP, thioesters with acyl carrier protein ACP4 354530-32-8DP, thioesters with acyl carrier protein ACP4 354530-33-9DP, thioesters with acyl carrier protein ACP4
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); RCT (Reactant); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent)
(kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT 106588-64-1 106588-70-9 209671-28-3 209671-29-4
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); RCT (Reactant); BIOL (Biological study); PROC (Process); RACT (Reactant or reagent)
(kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT 354760-45-5P 354760-46-6P 354760-47-7P 354760-48-8P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

IT 79956-01-7, **Polyketide synthase**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(modules; kinetic channeling of intermediates between **polyketide synthase** modules in deoxyerythronolide B synthase)

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:698890 HCAPLUS
DOCUMENT NUMBER: 134:14960
TITLE: The expansion of mechanistic and organismic diversity associated with non-ribosomal peptides
AUTHOR(S): Moffitt, M. C.; Neilan, B. A.
CORPORATE SOURCE: School of Microbiology and Immunology, University of New South Wales, Sydney, 2052, Australia
SOURCE: FEMS Microbiology Letters (2000), 191(2), 159-167
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 73 refs. Non-ribosomal peptides are a group of secondary metabolites with a wide range of bioactivities, produced by prokaryotes and lower eukaryotes. Recently, non-ribosomal synthesis has been detected in diverse microorganisms, including the myxobacteria and cyanobacteria. Peptides biosynthesized non-ribosomally may often play a primary or secondary role in the producing organism. Non-ribosomal peptides are often small in size and contain unusual or modified amino acids. Biosynthesis occurs via large modular **enzyme** complexes, with each module responsible for the activation and thiolation of each amino acid, followed by peptide bond formation between activated amino acids. Modules may also be responsible for the **enzymic** modification of the **substrate** amino acid. Recent anal. of biosynthetic gene clusters has identified novel integrated, mixed and hybrid **enzyme** systems. These diverse mechanisms of biosynthesis result in the wide variety of non-ribosomal peptide structures and bioactivities seen today. Knowledge of these biosynthetic systems is rapidly increasing and methods of genetically engineering these systems are being developed. In the future, this may lead to rational drug design through **combinatorial biosynthesis** of these **enzyme** systems.
CC 10-0 (Microbial, Algal, and Fungal Biochemistry)
ST review microorganism **nonribosome** peptide
IT Microorganism
(expansion of mechanistic and organismic diversity assocd. with **nonribosomal** peptides in microbes)
IT Peptides, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(**nonribosomal**; expansion of mechanistic and organismic diversity assocd. with **nonribosomal** peptides in microbes)
REFERENCE COUNT: 73 THERE ARE 73 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 9 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:332816 HCAPLUS
TITLE: Investigating the **combinatorial biosynthetic** potential of a modular **polyketide synthase** system: In vitro **substrate** specificity studies on individual modules of 6-deoxyerythronolide B synthase.
AUTHOR(S): Wu, Nicholas; Khosla, Chaitan
CORPORATE SOURCE: Department of Chemistry, Stanford University, Stanford, CA, 94305, USA

SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), ORGN-689. American Chemical Society: Washington, D. C.
CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB **Polyketide synthases** have drawn much attention in recent years because of their immense potential for the **combinatorial biosyntheses** of complex mols. The realization of this potential is dependent on, among other things, the ability of these **enzymes** to incorporate and process unnatural **substrates**. It has been previously shown through in vivo studies that the 6-deoxyerythronolide B synthase (DEBS) system possesses remarkable tolerance for unnatural **substrates**. In this study, we have examd. more closely the **substrate** specificity of individual DEBS modules through steady state kinetics measurements using a variety of synthetic **substrates**.

L23 ANSWER 10 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:673723 HCAPLUS
DOCUMENT NUMBER: 132:10185
TITLE: Initiation, elongation, and termination strategies in polyketide and polypeptide antibiotic biosynthesis
AUTHOR(S): Keating, Thomas A.; Walsh, Christopher T.
CORPORATE SOURCE: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, USA
SOURCE: Current Opinion in Chemical Biology (1999), 3(5), 598-606
CODEN: COCBF4; ISSN: 1367-5931
PUBLISHER: Current Biology Publications
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 42 refs. focused on **polyketide synthases** and **nonribosomal peptide synthases**. Progress in sequence anal. of biosynthetic gene clusters encoding polyketides and **nonribosomal** peptides and in the reconstitution of in vitro activities continues to reveal new insights into the growth of these natural products' acyl chains, which have been revealed as a series of elongating, covalent, acyl **enzyme** intermediates on their multimodular scaffolds. Studies that focus on the three stages of natural product biosynthesis - initiation, elongation, and termination - have yielded crucial information on monomer **substrate** specificity, domain and module portability, and product release mechanisms, all of which are important not only for an understanding of this exquisite **enzymic** machinery, but also for the rational construction of new, functional synthetases and synthases that are a goal of **combinatorial biosynthesis**.
CC 7-0 (Enzymes)
Section cross-reference(s): 6, 10
ST review **polyketide nonribosomal peptide synthase**; antibiotic polyketide polypeptide biosynthesis review
IT 79956-01-7, **Polyketide synthase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(initiation, elongation, and termination strategies in polyketide and polypeptide antibiotic biosynthesis)

IT 115288-50-1, **Peptide synthase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(**nonribosomal**; initiation, elongation, and termination strategies in polyketide and polypeptide antibiotic biosynthesis)
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 11 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1998:624768 HCAPLUS
DOCUMENT NUMBER: 130:22144
TITLE: Characterization of the macrolide P-450 hydroxylase from Streptomyces venezuelae which converts narbomycin to picromycin
AUTHOR(S): Betlach, Melanie C.; Kealey, James T.; Betlach, Mary C.; Ashley, Gary W.; McDaniel, Robert
CORPORATE SOURCE: KOSAN Biosciences Inc., Burlingame, CA, 94010, USA
SOURCE: Biochemistry (1998), 37(42), 14937-14942
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The post-**polyketide synthase** (PKS) biosynthetic tailoring of macrolide antibiotics usually involves one or more oxidn. reactions catalyzed by cytochrome P 450 monooxygenases. As the specificities of members from this class of **enzymes** vary significantly among PKS gene clusters, the identification and study of new macrolide P450s are important to the growing field of **combinatorial biosynthesis**. We have isolated the cytochrome P 450 gene picK from Streptomyces venezuelae which is responsible for the C-12 hydroxylation of narbomycin to picromycin. The gene was located by searching regions proximal to modular PKS genes with a probe for macrolide P 450 monooxygenases. The overprodn. of PicK with a C-terminal six-His affinity tag (PicK/6-His) in Escherichia coli aided the purifn. of the **enzyme** for kinetic anal. PicK/6-His was shown to catalyze the in vitro C-12 hydroxylation of narbomycin with a kcat of 1.4 s-1, which is similar to the value reported for the related C-12 hydroxylation of erythromycin D by the EryK hydroxylase. The unique specificity of this **enzyme** should be useful for the modification of novel macrolide **substrates** similar to narbomycin, in particular, ketolides, a promising class of semisynthetic macrolides with activity against erythromycin-resistant pathogens.
CC 7-5 (Enzymes)
Section cross-reference(s): 3, 10
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L23 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:283806 HCAPLUS
DOCUMENT NUMBER: 126:314085
TITLE: Gain-of-Function Mutagenesis of a Modular **Polyketide Synthase**
AUTHOR(S): McDaniel, Robert; Kao, Camilla M.; Fu, Hong; Hevezi, Peter; Gustafsson, Claes; Betlach, Mary; Ashley, Gary; Cane, David E.; Khosla, Chaitan
CORPORATE SOURCE: KOSAN Biosciences Inc., Burlingame, CA, 94010, USA
SOURCE: Journal of the American Chemical Society (1997),

119(18), 4309-4310
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Modular **polyketide synthases** (PKSs) are multifunctional **enzyme** assemblies that catalyze the biosynthesis of numerous structurally complex natural products such as erythromycin, avermectin, and rapamycin. Active sites are clustered in "modules" that each perform a single cycle of condensation and .beta.-ketoredn. in polyketide biosynthesis. Whereas the feasibility of loss-of-function mutagenesis of modular PKSs has been repeatedly demonstrated, gain-of-function mutagenesis of modular PKSs, until now, has not been realized. The latter is particularly challenging since, in addn. to recognition of an unnatural **substrate**, the newly introduced activity must compete with chain transfer and/or release. Using a recently established screening system for the introduction of DH (dehydratase) activity into the reductive segment of module 2, the authors show that the reductive segment from module 4 of the rapamycin PKS can catalyze the formation of the expected dehydrated triketide intermediate. Furthermore, this **enzyme**-bound intermediate is faithfully processed by the next module of the erythromycin PKS with undiminished efficiency in vivo. In addn. to expanding the potential of modular PKSs for **combinatorial biosynthesis**, the introduction of a functional dehydratase (DH) domain into module 2 of the complete erythromycin PKS could facilitate convenient access to the ketolides, a recently discovered class of erythromycin derivs. with broad spectrum antibacterial activity against a variety of clin. important susceptible and resistant organisms.

CC 7-5 (Enzymes)
Section cross-reference(s): 1

ST mutagenesis **polyketide synthase** dehydratase domain; rapamycin synthase dehydratase domain engineering; antibacterial ketolide prepn engineered **polyketide synthase**

IT **Enzyme** functional sites
(active; introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT Genetic engineering
Mutagenesis
(introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT Polyketides
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT Antibiotics
(ketolides; introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT 79956-01-7P, **Polyketide synthase** 128172-72-5P,
Erythromycin **polyketide synthase** 175449-84-0P,
Rapamycin synthase
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); CAT (Catalyst use); PRP (Properties); BIOL (Biological study); PREP (Preparation); USES (Uses)
(introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT 181481-82-3P 187460-57-7P 189325-67-5P 189325-68-6P
RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

IT 9044-86-4P, Dehydratase
RL: BPN (Biosynthetic preparation); CAT (Catalyst use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(introduction of rapamycin **polyketide synthase** (PKS) dehydratase domain into module 2 of erythromycin PKS results in a PKS capable of completely processing a regiospecifically dehydrated triketide intermediate)

L23 ANSWER 13 OF 13 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1996:157011 HCAPLUS
DOCUMENT NUMBER: 124:224696
TITLE: Engineered biosynthesis of novel polyketides: properties of the whiE aromatase/cyclase
AUTHOR(S): Alvarez, Miguel A.; Fu, Hong; Khosla, Chaitan; Hopwood, David A.; Bailey, James E.
CORPORATE SOURCE: Inst. Biotechnol., ETH, Zurich, CH-8093, Switz.
SOURCE: Nature Biotechnology (1996), 14(3), 335-8
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The ORFVI from the whiE cluster of genes which is responsible for the biosynthesis of the Streptomyces coelicolor spore pigment has been described as a bifunctional aromatase/cyclase. In order to evaluate its potential use for generating novel polyketides, combinations of this gene with those encoding minimal **polyketide synthase** **enzymes** with or without a ketoreductase from S. coelicolor A3(2) were constructed and analyzed in vivo. Anal. of the polyketide products generated from these constructs indicates that the whiE-ORFVI **enzyme** has properties similar to those of TcmN, although the whiE aromatase/cyclase normally acts on a polyketide intermediate that is 4 carbons longer than the TcmN **substrate**. The whiE aromatase/cyclase can influence the regiospecificity of the first cyclization of unreduced, but not reduced, backbones and is also responsible for the second ring aromatization. An unusual new polyketide, EM18, was identified which is not seen in equiv. strains expressing the tcmN aromatase/cyclase or the act aromatase genes. The structure of EM18 suggests that the whiE-ORFVI product might have some unique properties within this family of **polyketide synthase** subunits,

and may therefore be useful in the design of **combinatorial biosynthetic** strategies.

CC 7-3 (Enzymes)
Section cross-reference(s): 3, 10

IT 9039-48-9D, Aromatase, bifunctional **enzyme** with polyketide cyclase 160995-36-8D, Gene actIV cyclase, bifunctional **enzyme** with aromatase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); CAT (Catalyst use); BIOL (Biological study); USES (Uses)
(engineered biosynthesis of polyketides by recombinant whiE aromatase/cyclase from Streptomyces coelicolor)